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# Identification of Ammonia as an Important Sediment-Associated Toxicant in the Lower Fox River and Green Bay, Wisconsin

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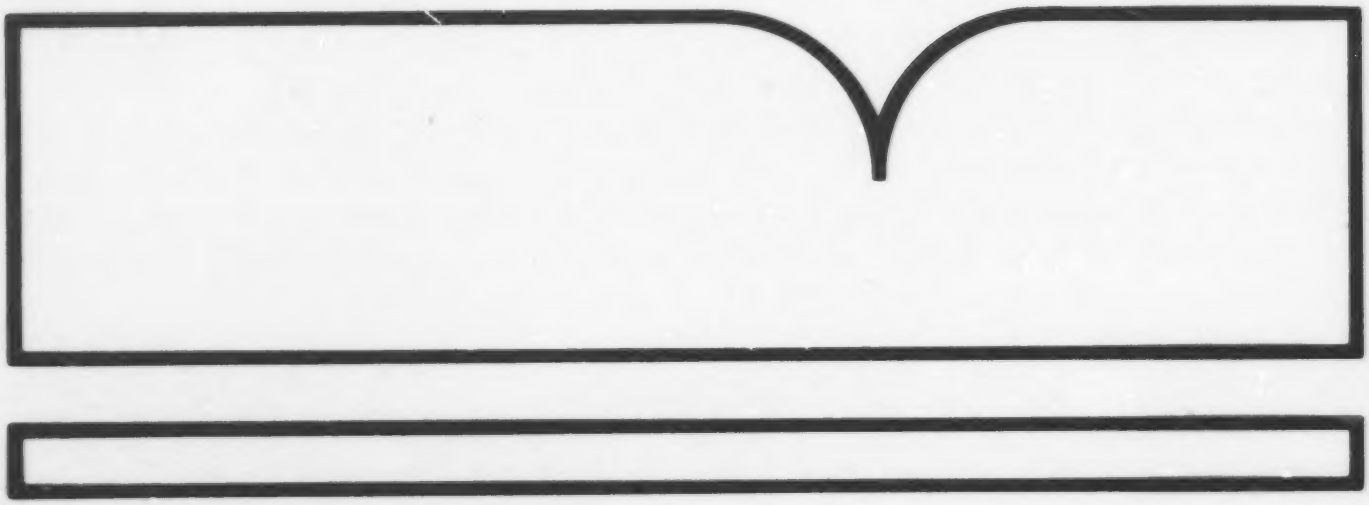

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# IDENTIFICATION OF AMMONIA AS AN IMPORTANT SEDIMENT-ASSOCIATED TOXICANT IN THE LOWER FOX RIVER AND GREEN BAY, WISCONSIN

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**Abstract**—Toxicity of sediment pore water from 13 sites in the lower Fox River/Green Bay watershed was assessed using a number of test species. Sediment pore water from the 10 lower Fox River sites exhibited acute toxicity to fathead minnows (*Pimephales promelas*) and *Ceriodaphnia dubia*, and pore water samples from all 13 sites were chronically toxic to *C. dubia*. Sediment pore water from seven of the sampling sites was toxic to *Selenastrum capricornutum*, but none of the samples were toxic to *Photobacterium phosphoreum*. Toxicity characterization, identification and confirmation procedures indicated that a significant amount of the acute toxicity of the pore water to fathead minnows and *C. dubia* was due to ammonia. The identification of ammonia, a naturally occurring compound in sediments, as a potentially important sediment-associated toxicant has implications for sediment toxicity assessment and control, not only in the Fox River and Green Bay, but in other freshwater and marine systems as well.

**Keywords**—Sediments Pore water Toxicity Ammonia

## INTRODUCTION

The presence of high concentrations of organic and inorganic contaminants in sediments throughout the Great Lakes and associated connecting channels has been well documented [1–6]. Other studies have demonstrated that sediments from different locations in the Great Lakes exhibit acute and chronic toxicity to a variety of aquatic organisms [7–11]. However, other than anecdotal accounts of the types and concentrations of contaminants present in the sediments, little has been established linking specific contaminants to observed toxicity. Because of the complexity of contaminant mixtures present in most toxic sediments, as well as uncertainties concerning the effects of sediment matrices on contaminant bioavailability, it has been exceedingly difficult to discern which compound(s) cause toxicity. Yet, this type of chemical-specific information may be critical if remediation addressing toxic sediments is to succeed.

Recently, several guidance documents addressing the problem of identifying specific toxic compounds in complex effluents were issued [12–14]. These procedures use toxicity-based fractionation schemes to characterize and identify compounds in aqueous samples exhibiting acute toxicity to aquatic organisms, and are specifically designed to address multiple toxicant interactions, as well as matrix effects on contaminant toxicity. A major strength of these toxicity identification schemes is that they enable direct relationships to be established between toxicity tests and analytical outputs, thereby simplifying the identification of specific toxicants in complex mixtures of compounds. Phase I of the approach consists of methods to remove, and/or render biologically unavailable, specific classes of compounds prior to toxicity testing [12]. Upon successful completion of Phase I, toxic compounds can be tentatively classified as polar or nonpolar organics, cationic metals, volatile compounds, oxidants and/or compounds whose toxicity is pH-dependent. Phase II procedures [13] utilize information from Phase I tests to focus on appropriate analytical techniques for fractionation and identification of suspect toxicants, and Phase III [14] consists of procedures designed to ensure that the suspect toxicants are the true toxicants.

The objectives of this study were twofold. The first was to evaluate the acute and chronic toxicities of sediments from several sites in the lower Fox River/Green Bay watershed using four test species: fathead minnows (*Pimephales promelas*), *Cerio-*

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The use of tradenames does not constitute endorsement by the U.S. Environmental Protection Agency.

*daphnia dubia*, *Selenastrum capricornutum* and *Photobacterium phosphoreum* (Microtox®). The lower Fox River/Green Bay system has been heavily impacted by pollutant inputs from both point and nonpoint sources. Specific contaminants known or suspected to be present at high concentrations in the system include: polychlorinated biphenyls, polycyclic aromatic hydrocarbons, resin acids, heavy metals and certain pesticides [15–17]. The second objective of the study was to use the toxicity identification techniques described above to identify specific compounds responsible for toxicity to one or more of the test species. The sediment fraction chosen for toxicity tests and identification procedures was interstitial (pore) water. Previous studies have demonstrated that toxicity and bioaccumulation of sediment-associated contaminants such as cadmium, zinc, mercury, kepone, fluoranthene and various organochlorines, such as chlorobenzenes, by benthic macroinvertebrates are highly correlated with the concentrations of these chemicals in pore water [18–22]. Although there is uncertainty as to the most appropriate manner with which to obtain a representative pore water sample, it still has been recognized as an appropriate fraction for the consideration of sediment toxicity [23,24].

## MATERIALS AND METHODS

### *Sediment collection and sample preparation*

Approximately 320 liters of sediment were collected, using an Eckman dredge, from each of 13 sites in the lower Fox River/Green Bay system in June and July 1988. Water samples, from 4 to 20 cm above the sediment/water interface, were collected at the same time from each site. Sites 1 to 10 were located along the Fox River, sites 11 and 13 were in Green Bay and site 12 (intended as a river reference site) was located in the East River, a tributary of the Fox River (Fig. 1). Samples were collected from depths ranging from approximately 1 to 8 m of water, and the sediments were collected from depositional zones at the river sites. Organic carbon content of sediments, as determined using

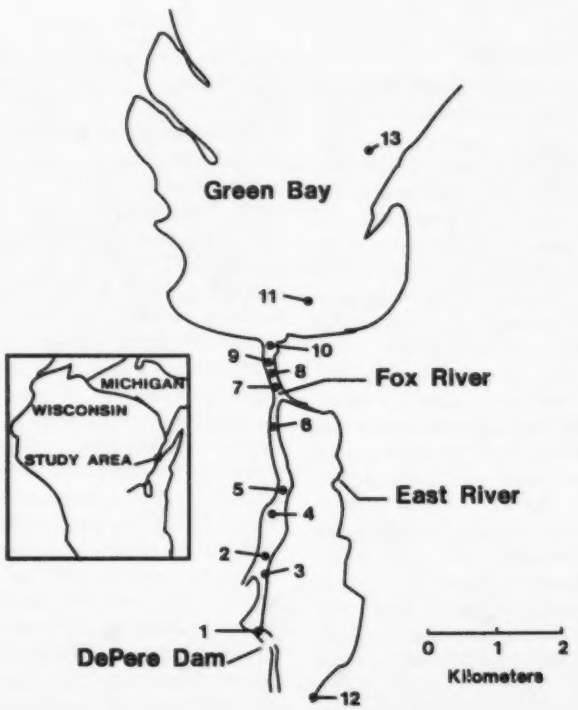


Fig. 1. Sampling locations in the lower Fox River/Green Bay watershed. The width of the Fox River is exaggerated relative to that of Green Bay.

a Dohrmann DC183 organic carbon analyzer, ranged from 1.3 to 8.3% at the different study sites (K. Lodge, University of Minnesota at Duluth, Duluth, MN, personal communication). Samples were placed in prerinsed plastic pails and shipped under refrigeration to the Environmental Research Laboratory at Duluth, where they were homogenized and stored at 4°C in the plastic pails (K. Lodge, University of Minnesota at Duluth, Duluth, MN, personal communication). All toxicity tests and toxicity identification experiments with the original 13 sediment samples were performed within a time period ranging from 3 to 16 weeks after collection of the sediments. A second sampling trip, to a subset of the original 13 sites, was made in September 1988. To confirm that observed sediment toxicity was not artifactual (i.e., related to storage conditions and time), sediment pore water was prepared on the same day these samples were collected, and toxicity tests were conducted within 4 d of sample collection.

Pore water was prepared by centrifugation of 250-ml aliquots of sediment for 45 min at 4,000 *g* in a refrigerated centrifuge. After preparation, particulate matter in the samples was allowed to settle for at least 24 h prior to decanting pore water for toxicity tests. Pore water was stored at 4°C, generally for less than 72 h, before testing.

*Toxicity tests*

Four-day acute toxicity tests with <24-h-old fathead minnow larvae were conducted using documented procedures [12,25]. Tests were performed at 25°C in duplicate 30-ml chambers, containing five fish per chamber, at pore water concentrations of 0, 6.25, 12.5, 25, 50 and 100%. The control/dilution water used for all experiments with fathead minnows and *C. dubia* was 10% mineral water (Perrier®, Vergeze, France) in ultrapure distilled, deionized water. Characteristics of the control/dilution water were: alkalinity, 28 mg/L as $CaCO_3$; hardness, 40 mg/L as $CaCO_3$; pH, 8.0.

Seven-day chronic toxicity tests with the cladoceran, *C. dubia*, were performed using standard procedures [26]. Tests were conducted at 25°C and were initiated with <8-h-old organisms. Assays were performed with six replicates at pore water concentrations of 0, 12.5, 25, 50 and 100%, and were renewed every other day.

Toxicity tests with the green algae, *S. capricornutum*, were performed using standard methods [26,27]. Tests were conducted in triplicate at pore water concentrations of 1, 24, 56, 75 and 100%. The control/dilution water consisted of freshwater algal medium to which 100 μg/L EDTA was added. Cell concentration in the initial inoculum of the test cultures was 10,000 cells/ml, and test containers were kept under a constant illumination of 400 ft-c at 24°C. Algal responses were determined with an electronic particle counter after 7-d exposures.

Microtox® toxicity tests were conducted using previously described procedures [11]. Tests were performed in duplicate, at pore water concentrations of 0, 7.5, 15, 30 and 60%. Bioluminescence readings were taken 15 min after initiation of the test.

*Toxicity identification procedures*

Pore water samples exhibiting acute toxicity to fathead minnows and *C. dubia* were subjected to the toxicity identification procedures developed for complex effluents [12–14]. Patterns in pore water toxicity to fathead minnows and *C. dubia* were similar (see Results). Therefore, to minimize sample volumes, *C. dubia* were used for the toxicity identification experiments. In these tests, five animals per replicate were exposed for 48 h in 10-ml volumes. Phase I characterization of several of the lower Fox River pore water samples indicated that acute toxicity was markedly decreased at a lower test pH, so Phase II identification was initially focused on determination of ammonia. Ammonia concentrations were measured using a specific ion electrode [28; Orion Research Inc., Cambridge, MA]. Phase III toxicity confirmation procedures included: (a) correlation of toxicity with ammonia concentration, (b) removal of ammonia via a zeolite cation exchange resin (Argent Chemical Laboratories, Redmond, WA) and (c) evaluation of relative species sensitivity.

*Statistical analyses*

Point estimates of the pore water concentration causing a 50% reduction in *C. dubia* fecundity or *S. capricornutum* biomass were calculated using a nonparametric monotonic regression technique [26,29]. The trimmed Spearman–Karber method was used to calculate LC50 values for the 48-h *C. dubia* and 96-h fathead minnow toxicity tests [30]. Pore water concentrations resulting in inhibition of bioluminescence of *P. phosphoreum* were evaluated using a linear regression package available from the manufacturers of Microtox® (Microbics, Inc., Carlsbad, CA). Relationships between acute or chronic toxicity and ammonia concentrations were evaluated with Pearson pairwise correlations, calculated using a standard statistical package [31].

## RESULTS

### *Sediment toxicity*

Analysis of benthic macroinvertebrates present in sediments from the various study sites revealed that all the sampling locations, with the exception of the East River (site 12), were characterized by very low diversity (M. Balcer, University of Wisconsin, Superior, WI, personal communication). The only organisms consistently present were oligochaetes and chironomids. In general, relative abundance and biomass of the oligochaetes and chironomids were considerably greater in sediments from the two Green Bay locations (sites 11 and 13) and the East River location (site 12) than in sediments from the 10 sites on the lower Fox River.

Toxicity tests with water overlying the sediments at the various study sites indicated an absence of acute toxicity to fathead minnows or acute or chronic toxicity to *C. dubia* (data not shown); however, pore water samples from all 13 of the sites were toxic to one or both of these species. Sediment pore waters from the 10 lower Fox River sites all exhibited some degree of acute toxicity to fathead minnows, with the greatest mortality occurring in samples of pore water from sites 2, 5, 6, 9 and 10 (Table 1). No mortality was observed in pore water from sites 11, 12 and 13.

Although *C. dubia* were less sensitive to the acute effects of the pore water than the fathead minnows, patterns in toxicity were generally the same (Table 1). Exposure to pore water from all 10 of the lower Fox River sites resulted in some degree of lethality to *C. dubia* within 48 h, with samples from sites 2, 5, 6, 9 and 10 producing the greatest mortality. As with the fathead minnows, sediment pore water from sites 11, 12 and 13 was not lethal to *C. dubia*. Pore water from all 13 of the sampling sites resulted in some degree of chronic toxicity to *C. dubia* (Table 1). Sites 2, 5, 6, 9 and 10 exhibited the greatest toxicity, and sites 11 and 13 the least.

Sediment pore water from sites 2, 5, 6, 7, 9, 10 and 13 caused more than a 50% inhibition of *S. capricornutum* biomass production, with the greatest inhibition occurring at site 13 (Table 1). Pore water from three of the other six sites slightly reduced growth, while pore water from the remaining three sites markedly stimulated growth (data not shown).

None of the pore water samples caused a consequential decrease in bioluminescence of *P. phosphoreum* (Table 1). Pore water from 10 of the 13 sites stimulated bacterial light production, and samples from the other three sites (2, 10 and 12), produced only a very minor inhibition of bioluminescence, i.e., 3 to 5% (data not shown).

Table 1. Toxicity of sediment pore waters from 13 sites in the lower Fox River/Green Bay watershed to fathead minnows, *C. dubia*, *S. capricornutum* and *P. phosphoreum*

| | Fathead minnow | *C. dubia* | | *S. capricornutum* | *P. phosphoreum* |
|---|---|---|---|---|---|
| Site | 96-h LC50 | 48-h LC50 | 168-h EC50[a] | 168-h EC50[b] | 15-min EC20[c] |
| 1 | 40.6 (34.1–48.4) | >100 | 46.7 (43.6–48.5) | >100 | >100 |
| 2 | 30.9 (25.3–37.8) | 63.0 (51.0–77.8) | 37.8 (37.5–38.4) | 81.0 (78.5–83.2) | >100 |
| 3 | 35.4 (NC[d]) | >100 | 60.3 (51.8–69.7) | >100 | >100 |
| 4 | 35.4 (NC) | 84.1 (NC) | 49.9 (45.3–60.6) | >100 | >100 |
| 5 | 18.0 (NC) | 56.1 (43.0–73.3) | 37.5 (37.4–38.5) | 69.6 (69.1–70.2) | >100 |
| 6 | 21.8 (17.8–26.6) | 39.7 (32.1–49.0) | 35.7 (34.4–36.6) | 82.5 (81.5–83.5) | >100 |
| 7 | 35.4 (NC) | 84.1 (NC) | 54.2 (47.2–63.4) | 89.2 (88.3–89.8) | >100 |
| 8 | 37.9 (30.4–47.2) | 75.8 (NC) | 73.7 (70.0–75.5) | >100 | >100 |
| 9 | 17.4 (NC) | 44.5 (34.1–58.2) | 29.3 (26.8–31.9) | 64.2 (62.2–66.5) | >100 |
| 10 | 21.1 (16.8–26.5) | 39.7 (32.1–49.0) | 31.9 (25.1–36.0) | 80.1 (78.3–81.8) | >100 |
| 11 | NM[e] | NM | 77.1 (73.7–80.1) | >100 | >100 |
| 12 | NM | NM | 53.8 (43.9–74.0) | >100 | >100 |
| 13 | NM | NM | 86.5 (72.2–93.2) | 52.8 (50.9–55.0) | >100 |

LC50, EC50 and EC20 values are expressed in percent pore water. The 95% confidence limits are indicated in parentheses.

[a]Concentration resulting in 50% reduction in reproduction.

[b]Concentration resulting in 50% reduction in biomass.

[c]Concentration resulting in 20% inhibition of light production.

[d]NC, reliable confidence limits could not be calculated due to lack of partial mortality.

[e]NM, no mortality.

*Toxicity identification*

Characterization of the toxicity of pore water from four of the sites exhibiting the greatest degree of lethality to fathead minnows and *C. dubia* indicated that the toxicity was highly pH-dependent. *C. dubia* were exposed to pore water from sites 5, 6, 9 and 10 that had been adjusted to a pH of 6.5 or 8.5. At the lower pH, no mortality was observed, while at pH 8.5 48-h LC50 values were slightly lower than those found in initial exposures (Table 2). Although a number of ionic compounds theoretically can be more toxic at high pH values than at low, an extremely common aquatic toxicant that exhibits this property is ammonia. Measurement of ammonia in the pore water samples revealed total concentrations in excess of 40 mg/L at sites 2, 5, 6, 9 and 10 and concentrations greater than 1 mg/L at all 13 of the sampling locations (Table 3).

When acute and chronic toxicities of the pore water to fathead minnows and *C. dubia* were correlated with total ammonia concentration in the samples, the resulting correlation coefficients were quite high, i.e., $r \geq 0.90$ (Fig. 2a–c). Correlations were made using total, rather than un-ionized, ammonia because pH of the pore waters drifted upward over the course of the toxicity tests, making it impossible to select a stable and representative pH for calculation of un-ionized ammonia concentrations. However, initial and final pH values for the various pore water samples were similar (approximately 7.8 and 8.4, respectively) and drifted in the same manner during the tests, so it is justifiable to make comparisons based on total ammonia. The mean pH of the toxicity tests with the fathead minnows and *C. dubia* (i.e., the mean of the initial and ending pore water pH values) was approximately 8.2, which would result in concentrations of un-ionized ammonia ranging from 1.3 to 4.4 mg/L at sites exhibiting acute toxicity [32]. These concentrations are within the range necessary to result in acute toxicity to fathead minnows and *C. dubia*. The 48-h and 96-h LC50 values for un-ionized ammonia for fathead minnows and *C. dubia*, tested under conditions similar to those in the present study, are 1.04 and 2.88 mg/L, respectively (J. Lazorchak, U.S. Environmental Protection Agency, Newtown Laboratory, Cincinnati, OH, personal communication).

In another confirmation experiment, pore water samples from the 10 lower Fox River sites were passed over a zeolite resin and tested for acute tox-

Table 2. Toxicity of sediment pore waters from four sites in the lower Fox River to *C. dubia* at pH 6.5 and pH 8.5

| | pH | |
|---|---|---|
| Site | 6.5 | 8.5 |
| 5 | NM[a] | 46.7 (34.4–63.2) |
| 6 | NM | 35.4 (NC[b]) |
| 9 | NM | 40.6 (31.7–52.0) |
| 10 | NM | 40.6 (31.7–52.0) |

LC50 values are for 48-h exposures and are expressed as percent pore water. The 95% confidence limits are indicated in parentheses.
[a]NM, no mortality.
[b]NC, reliable confidence limits could not be calculated due to lack of partial mortality.

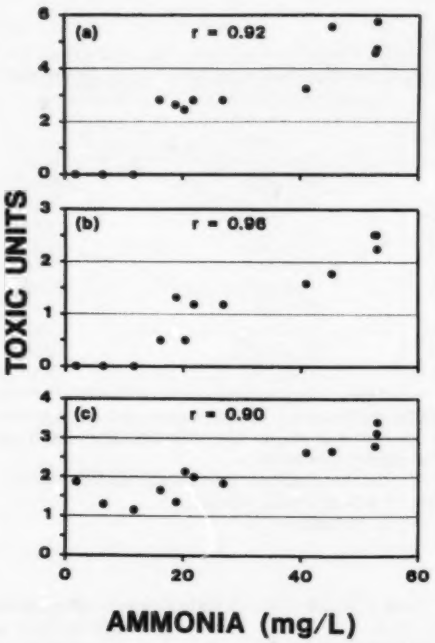


Fig. 2. Correlation of concentrations of ammonia in sediment pore waters from the lower Fox River/Green Bay watershed with toxicity of the samples to fathead minnows and *C. dubia*. Toxicity is expressed as toxic units (i.e., 100/LC50 or 100/EC50), for: (a) 96-h fathead minnow mortality, (b) 48-h *C. dubia* mortality and (c) 168-h *C. dubia* reproduction. When no mortality was observed in the 96-h fathead minnow or the 48-h *C. dubia* exposures, a value of zero toxic units was assigned. In the two instances in the 48-h *C. dubia* test in which less than 50% mortality occurred at a pore water concentration of 100%, a value of 0.5 toxic units was assigned for computational purposes.

Table 3. Ammonia concentrations in sediment pore waters from 13 sites in the lower Fox River/Green Bay watershed

| Site | Ammonia (mg/L) |
|---|---|
| 1 | 20.3 ± 1.1 |
| 2 | 40.9 ± 2.1 |
| 3 | 16.1 ± 1.4 |
| 4 | 21.8 ± 1.1 |
| 5 | 45.3 ± 2.5 |
| 6 | 52.6 ± 1.8 |
| 7 | 26.8 ± 1.0 |
| 8 | 18.8 ± 0.6 |
| 9 | 53.0 ± 3.2 |
| 10 | 52.9 ± 1.8 |
| 11 | 6.3 ± 0.7 |
| 12 | 1.8 ± 0.5 |
| 13 | 11.6 ± 1.5 |

Data are given as the $\bar{x} \pm$ SE for determinations on three separate pore water preparations.

Table 4. Toxicity of sediment pore waters from 10 sites in the lower Fox River to *C. dubia*, before and after being passed over a zeolite resin

| Site | Initial | Post-zeolite |
|---|---|---|
| 1 | 89.1 (NC[a]) | NM[b] |
| 2 | 53.6 (39.6–72.6) | NM |
| 3 | 70.7 (NC) | NM |
| 4 | 70.7 (NC) | NM |
| 5 | 35.4 (NC) | NM |
| 6 | 35.4 (NC) | NM |
| 7 | 70.7 (NC) | NM |
| 8 | >100 | NM |
| 9 | 35.4 (NC) | NM |
| 10 | 35.4 (NC) | NM |

LC50 values are for 48-h exposures and are expressed as percent pore water. The 95% confidence limits are indicated in parentheses.
[a]NC, reliable confidence limits could not be calculated due to lack of partial mortality.
[b]NM, no mortality.

icity to *C. dubia*. Passing the samples over the resin reduced ammonia concentrations to less than 1 mg/L in the samples, and also completely removed acute lethality at all 10 sites (Table 4).

To evaluate temporal variability in the toxicity of sediments from the lower Fox River, a second set of samples was collected in September 1988 from a subset of the original 13 sampling sites. Results obtained with the second set of samples corroborated our earlier observations. Pore water from sediments collected in September from sites 2, 6, 8 and 9 exhibited the same pattern of acute toxicity to fathead minnows and *C. dubia* as the June/July samples from these sites (Table 5). Ammonia concentrations in the September samples from sites 2, 3, 6 and 9 also exhibited a pattern similar to that seen in earlier samples (Table 5). Ammonia concentrations in the September samples, although high, were somewhat lower than those found in the June/July samples; however, due to the limited amount of sample volume from the second collection, it was impossible to determine whether this may have been related to analytical variability, sampling or storage time.

## DISCUSSION

Pore water from sediments collected at several sites in the lower Fox River and Green Bay exhibited acute or chronic toxicity to fathead minnows, *C. dubia* and *S. capricornutum*, but not to *P. phosphoreum*. Patterns in toxicity found with fathead minnows and *C. dubia* were virtually identical, while in some instances *S. capricornutum* were sensitive to factor(s) not affecting the animals (e.g., site 13). Toxicity identification indicated that a significant amount of the observed acute toxicity to fathead minnows and *C. dubia* was due to ammonia. Evidence supporting this conclusion included: (a) pore water toxicity was highly pH-dependent,

Table 5. Acute toxicities and ammonia concentrations of sediment pore waters, collected in September 1988, from five sites in the lower Fox River

| Site | Ammonia (mg/L) | Fathead minnow | *C. dubia* |
|---|---|---|---|
| 2 | 33.5 | 25.0 (20.1–31.1) | 40.6 (34.1–48.4) |
| 3 | 6.2 | ND[a] | ND |
| 6 | 39.2 | 16.5 (14.5–18.8) | 35.4 (NC[b]) |
| 8 | ND | 70.7 (NC) | >100 |
| 9 | 26.7 | 20.3 (17.0–24.2) | 37.9 (33.2–43.2) |

LC50 values for *C. dubia* are for 48-h exposures, and for fathead minnows are for 96-h exposures. Data are expressed in percent pore water. The 95% confidence limits are indicated in parentheses.
[a]ND, not determined.
[b]NC, reliable confidence limits could not be calculated due to lack of partial mortality.

with decreased toxicity at a lower pH, (b) concentrations of ammonia in the pore water samples were consistent with those known to result in acute toxicity to fathead minnows and *C. dubia*, (c) there were high correlations between toxicity of the pore water samples and ammonia concentration (i.e., $r \geq 0.90$), (d) removal of ammonia with a cation exchange resin resulted in complete removal of acute toxicity and (e) the relative sensitivity of three test species to the pore water samples closely paralleled their sensitivity to ammonia, i.e., fathead minnows were more sensitive than *C. dubia*, while *P. phosphoreum* were essentially unaffected [33; J. Lazorchak, U.S. Environmental Protection Agency, Newtown Laboratory, Cincinnati, OH, personal communication]. The identification of ammonia as a potentially important sediment-associated toxicant raises several questions concerning sediment toxicity assessment not only in the lower Fox River and Green Bay but in other systems as well.

Different studies have shown that sediments from anthropogenically impacted regions around the Great Lakes exhibit acute and chronic toxicity to a variety of aquatic organisms [7–11]. Therefore, it came as no surprise that sediment pore waters from the heavily polluted lower Fox River/Green Bay watershed were toxic. It was unexpected, however, that ammonia would play such a large role in determining sediment pore water toxicity in this system. The fact that sediments in certain types of systems can contain large concentrations of ammonia is well established [34]; however, relatively little is known concerning actual impacts of sediment ammonia in terms of toxicity to aquatic organisms. Studies by the U.S. Army Corps of Engineers have indicated that potentially toxic concentrations of ammonia can be released from dredged sediments [35]. Also, Jones and Lee [36] noted that of more than 30 contaminants measured in sediments from New York Harbor only concentrations of ammonia correlated with observed toxicity of the sediments to grass shrimp. Based on the results of these studies, as well as data from the present investigation, it is apparent that ammonia may be a relatively common sediment toxicant in both marine and freshwater systems.

Concentrations of ammonia in sediment pore water in the present study were similar to those that have been found at other sites in Green Bay (V. Klump, University of Wisconsin, Center for Great Lakes Research, Milwaukee, WI, personal communication). Thus, although the lower Fox River receives direct inputs of ammonia from sources such as sewage treatment plants and pulp and paper mills [37], given the uniformly high concentrations of ammonia in the sediments, it is possible that direct inputs are relatively less important than the amount of ammonia generated by natural processes. Because ammonia in most sediments is due to the natural degradation of nitrogen-containing organic compounds by microbes, and because it can accumulate to high concentrations under anaerobic conditions [34], it presents a unique challenge to researchers attempting to address the question of sediment toxicity. In some cases, the presence of high concentrations of ammonia may be due to anthropogenic sources, either through direct inputs or enrichment of the system by various nutrients, and thus needs to be dealt with in terms of point or nonpoint source control. In other instances, however, large concentrations of ammonia in sediments may be due only to natural inputs to a eutrophic system. Unfortunately, a significant amount of watershed-specific baseline data concerning sediment ammonia concentrations and ammonia and nutrient inputs would be needed to perform mass balance calculations on which to base these judgments.

The presence of ammonia can greatly complicate the interpretation of results of sediment toxicity tests. High concentrations of ammonia in sediments could mask the effects of other toxicants, and even small concentrations may conceivably interact with other sediment-associated contaminants in additive, synergistic or antagonistic fashions. It is clear that a consistent methodology needs to be developed to deal with the phenomenon of ammonia in sediment samples being tested for toxicity. First, it would be prudent to routinely measure ammonia in any sediments that may be expected to contain toxic concentrations. If ammonia is present, techniques useful for negating its toxic effects include: (a) manipulation of test pH to minimize the concentration of un-ionized ammonia; (b) removal of ammonia via cation exchange resins, such as zeolites; (c) removal of ammonia by air-stripping at a high pH; or (d) allowing ammonia to dissipate through nitrification processes by aerating the sediment sample for some period of time before toxicity testing. All of these techniques have inherent drawbacks in that toxic compounds other than ammonia could be removed or altered; however, used in conjunction with one another, they represent a rational approach for dealing with the confounding effects of ammonia toxicity in sediment samples. An alternative approach to dealing with high concentrations of sediment ammonia may be to use

a test species that is not sensitive to ammonia (e.g., *P. phosphoreum*); however, a species of this type also may be less sensitive to other types of sediment contaminants.

Little research has been conducted concerning the impact of specific sediment-associated contaminants on aquatic communities, and evaluation of the role of sediment ammonia as an influence on aquatic organisms may prove to be even more difficult than for other types of contaminants. One problem with assessing the effects of ammonia on aquatic ecosystems is that naturally occurring concentrations not only can vary seasonally, but also from year to year [34]. Also, the pH of sediments and overlying waters can vary markedly from season to season, thereby further complicating the interpretation of ammonia toxicity. Finally, because high concentrations of ammonia often occur in conjunction with anaerobic conditions, stress due to low concentrations of dissolved oxygen could well exacerbate the toxicity of ammonia to benthic organisms [38]. Considering all these factors, it is difficult to discern whether the relatively low species diversity and abundance observed in sediments from the lower Fox River may be related to ammonia. Because ammonia occurs naturally in certain systems, it is probable that some species of benthic organisms are relatively tolerant of moderate levels of ammonia. However, given the large concentrations of ammonia found in the lower Fox River sediments, it is reasonable to assume that certain benthic species might be adversely impacted. Also, fish species such as walleye, which are relatively sensitive to ammonia and also have certain life stages closely associated with benthic habitats (i.e., eggs), may be affected by large concentrations of ammonia in sediments. Moreover, the release of ammonia from sediments during resuspension events, such as dredging or storms, conceivably could impact upper-water column species [35].

Different approaches have been proposed to assess existing or potential impacts of contaminated sediments on aquatic ecosystems [39,40]. These approaches vary slightly, but they generally feature the use of toxicity tests and extensive chemical analyses, often in conjunction with evaluation of benthic communities. Although these types of data are valuable, if the major goal of remediation is to reduce sediment toxicity, it is imperative that those compound(s) responsible for toxicity be identified. For example, remedial plans for reducing concentrations of a toxicant such as ammonia would necessarily be quite different from those designed to deal with contaminants such as heavy metals. Thus, the application of some sort of toxicity identification process clearly could be critical to the success of sediment toxicity control programs.

It should be stressed that, although the toxicity identification procedures used in the present study proved to be useful for assessing the toxicity of pore water in sediment samples from the lower Fox River and Green Bay, they would not be useful in all situations. First, because the toxicity identification approaches used in this study are designed only for samples exhibiting acute toxicity, these procedures generally would not be useful for identifying chronically toxic contaminants in sediments. Also, although various studies suggest that pore water may be a suitable fraction for sediment toxicity testing [18–22], many types of exposures still need to be conducted using bulk sediments. This is particularly true in situations where benthic organisms may be exposed to toxicants via ingestion of contaminated particles. Therefore, toxicity identification schemes based only on aqueous sediment fractions cannot adequately assess all types of sediment toxicants.

In summary, sediment pore waters from several sites in the lower Fox River and Green Bay were toxic to a variety of aquatic organisms, with a significant amount of the toxicity due to ammonia. The identification of ammonia as an important sediment-associated toxicant has several implications for sediment toxicity assessment and control programs. These implications include questions concerning the identification and control of sources of ammonia and possible interactions of ammonia with other sediment toxicants. Finally, this study demonstrates how toxicity identification approaches can be applied to sediment toxicity assessment and suggests the need for routine use of some sort of toxicity identification process to ensure the success of sediment remediation programs.

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